

Toward Activatable Collagen Mimics: Combining DEPSI “Switch” Defects and Template-Guided Self-Organization to Control Collagen Mimetic Peptides

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Collagen mimetic peptides (CMPs), which imitate various structural or functional features of natural collagen, constitute advanced models illuminating the folding aspects of the collagen triple helix (CTH) motif. In this study, the CMPs of repeating Gly–Pro–Pro (GPP) triplets are tethered to an organic scaffold based on a tris(2-aminoethyl) amine (TREN) derivative (TREN–(suc–OH)₃). These three templated peptide strands are further expanded via native chemical ligation to increase the number of GPP triplets and lead to a TREN–(suc–GPPGPPG(Ψ)SPGPP–CPP[GPP]₄)₃ construct. The incorporation of an ester switch segment, G(Ψ)S, as a positional *O*-acyl isopeptide (DEPSI) defect into the peptide strands allows the pH-controlled acceleration of CTH formation. The strand assembly process is monitored by circular dichroism (CD) spectroscopy. The results of pH jump experiments and thermal denaturation studies provide new insights into the contributions of structural DEPSI defects to the template-guided self-assembly of the CTH motif. While the organic scaffold drives the CTH formation, the switch defects act as temporary opponents and slow down the folding. CD spectroscopy data confirm that the switch defects contribute to the formation of a more stable CTH motif by enhancing the structural dynamics at the early stage of the folding process.

insights into the hierarchical organization process of collagen have been gained.^[2,3] CMPs represent promising model systems that can elucidate the formation of a collagen triple helix (CTH), and their rod-like nanostructures can be used as hierarchical biomimetic materials.^[3–6] The synthetic imitation of collagen structures and materials has been extensively investigated, reaching from oligopeptides to peptide–polymer conjugates to de novo proteins.^[7–14] Natural collagen exhibits valuable properties such as good biocompatibility, uniform hierarchical organization, and high anisotropic tensile strength, which allow the successful utilization of collagen-based materials in tissue engineering, cosmetics, and pharmacology.^[4,15–19] However, various issues such as batch-to-batch variations and prion cross-contamination confined the benefits of natural collagen and stimulated research on synthetic collagen mimics.^[8]

While the biosynthesis and bioprocessing mechanism of collagen are investigated in great detail,^[20] the challenges related to its chemical synthesis originate from


1. Introduction

In the recent decades, collagen mimetic peptides (CMPs)^[1] have attracted considerable attention from researchers, and

the simplicity and perfection of the extraordinary protein. The primary collagen structure is composed of 1021 repeating Gly–Xaa–Yaa tripeptide units, where Xaa predominantly includes L-proline and Yaa mainly consists of L-hydroxyproline (Hyp). The individual chains form left-handed helices, three of which are associated to form a right-handed triple helix (CTH).^[21] Various strategies have been explored to simplify the collagen sequence,^[22] which included the assembly of discrete CTHs and suppression of ill-defined agglomeration. Hartgerink and co-workers utilized the intriguing effectiveness of native chemical ligation (NCL) to obtain high-molecular-weight collagen-like peptides by realizing the required amino acid sequence in the form of well-defined but not monodispersed strands.^[23] Elegantly, “sticky ends” or appropriate amino acid pairs have been implemented to achieve a more effective control over the CTH self-assembly process to produce materials with exceptional properties that were attributed to the internal hierarchical structure.^[24,25] Moreover, the side chain functionalization of CMPs exploited the γ -hydroxyl functionality of Hyp residues in the peptide strand. This enabled the stabilization of CTH by fluoro-substituents^[26] or the introduction of azido groups for “click” modifications.^[27,28] However, Gly–Pro–Pro (GPP) remains an important fundamental

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consensus sequence for the CMP investigation and reference for bacterial collagen-like proteins that do not contain post-translationally modified amino acids.^[29–31]

Among various methods,^[32,33] the utilization of synthetic scaffolds has become a promising strategy for templating and nucleating CTHs.^[7,34,35] A rich structural set of templates was developed for this purpose. For instance, Goodman et al. reported template-tethered homotrimeric CMPs that utilized tris(2-aminoethyl) amine (TREN) succinic acid derivatives to promote CTH formation.^[36,37] While templates can tether three CMP strands together and thus construct a permanently active assembly system, additional control mechanisms over the strand assembly seem to be mandatory. This can be realized by employing external triggers that allow advancing spontaneous assembly systems toward switchable/activable structures. Although the self-assembly of other strongly aggregating peptides can be controlled by performing minor pH changes, enzymatic processing, as well as increasing Ca^{2+} or Zn^{2+} levels,^[38–47] these strategies have not been applied to control the template-guided CTH formation. In particular, the *O*-acyl isopeptide (DEPSI) segment (referred to as “switch segment”^[48]) is capable of activating the self-assembly properties, e.g., of β -sheet forming peptides.^[48–50] The efficiency of the DEPSI method was demonstrated independently by Mutter et al.,^[48] Carpino et al.,^[51] and Kiso and co-workers.^[52] as, for instance, A β 1–42 amyloid peptides were synthesized cleanly, and self-assembly kinetics could be modulated via minor pH changes by implementing switch defects. Thus, the switch-controlled self-organization will be a promising tool to modulate the assembly in template-guided CMPs as the template drives CTH formation, while the switch defects act as antagonists.

Herein, we combined a template preorganization approach with the pH-triggered activation of a switch segment to control the formation of CTHs in CMPs (Figure 1).

2. Results and Discussion

Although the chemical synthesis of collagen-like peptides enables the incorporation of hydroxylated amino acid derivatives (such as Hyp residues), the repetitive unit [Gly–Pro–Pro] was used to allow a systematic comparison with the described collagen model strands.^[4,46,53,54] TREN possesses a suitable template structure for preorganizing CMP strands.^[37] In this study, TREN was reacted with succinic acid anhydride to produce a TREN template core (TREN – (suc – OH)₃; see the Supporting Information).

The native chemical ligation was employed to connect [GPP]_x peptides (an amine component) with the template (a carboxyl component). NCL represents a highly robust ligation methodology that tolerates various functionalities and requires the N-terminal introduction of a Cys residue to the peptide strands.^[23,55] For effective ligation, a thioester component is attached to the template side. NCL can be considered a critical step in the synthesis process because of the steric restrictions of coupling three peptide strands to the central TREN core. Taking this into account, it was expectable that the NCL of CPP[GPP]₄ strands to a TREN-(suc-thioester)₃ template never leads to the formation of a desired product (see the Supporting Information). Despite intensive efforts to optimize the reaction conditions, not the desired product but templates with two peptide strands and a cyclic cross-link were detected as side products by ultraperfor-

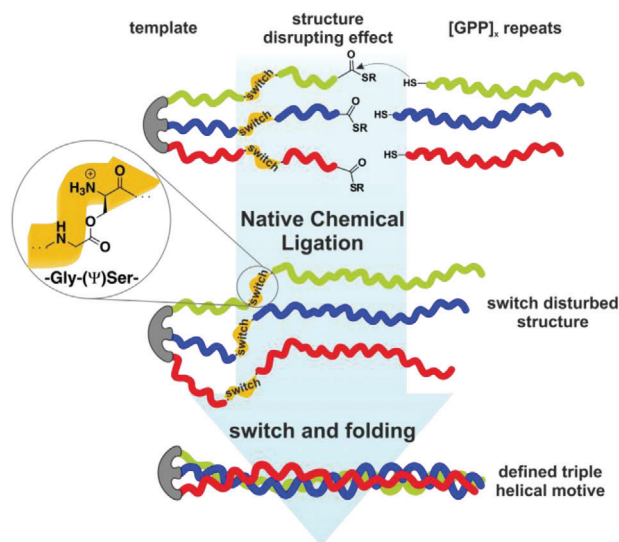
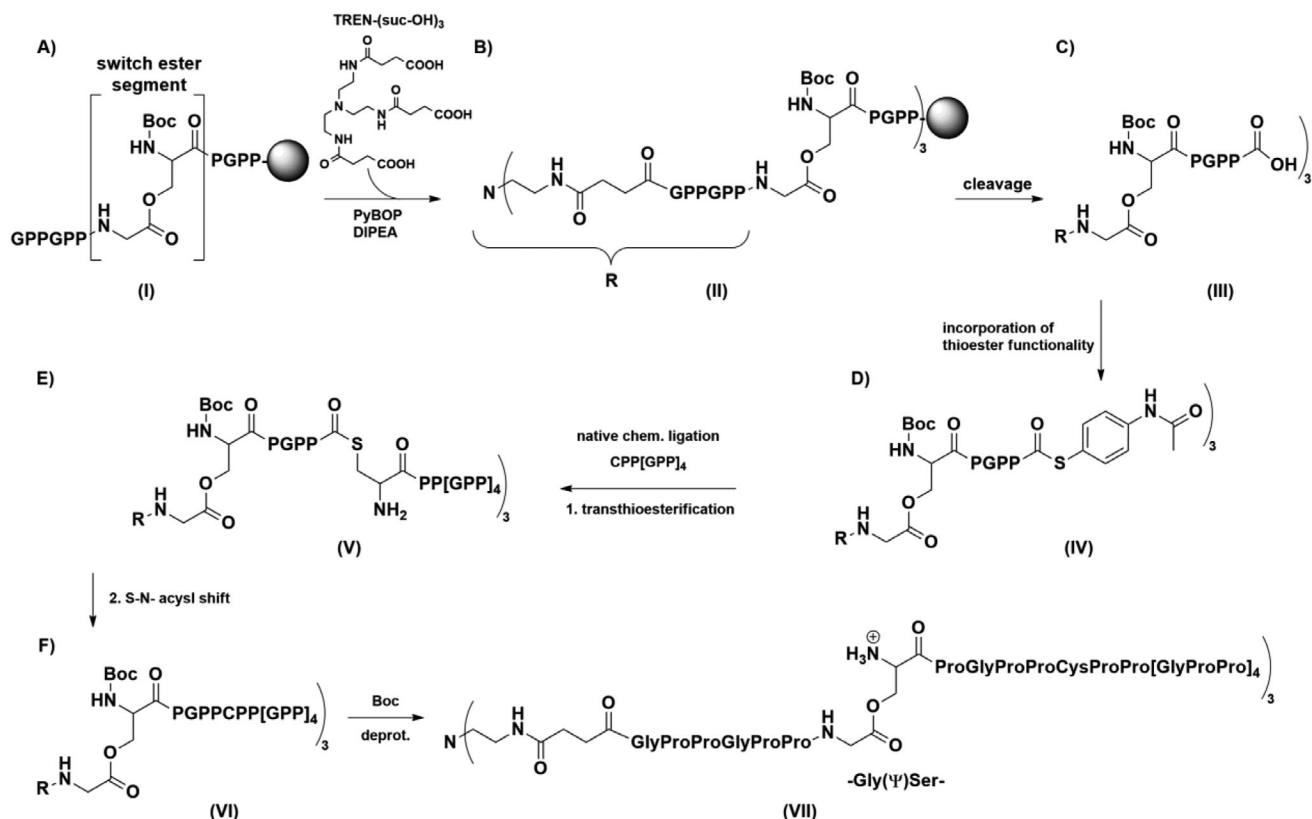


Figure 1. Switchable template for controlling the CMP self-assembly into triple helix motifs. The utilized synthesis strategy involved the fusion of the template that included the structure-disrupting switch segments (top, left) with collagen mimetic [GPP]_x peptides (top, right) via NCL. The obtained template-tethered homotrimeric CMPs contained three switch defects, which reduce aggregation tendency (middle) and activation of the self-assembly process due to minor pH changes, resulting in CTH formation preorganized by the TREN template (bottom).

mance liquid chromatography–mass spectrometry (UPLC–MS). This might be rationalized by the fact that the ligation of the third peptide strand to the core containing two peptides is rather slow. Moreover, the β -thiol side-chain functionalities of the Cys residues formed by attached peptide strands are located close to the third thioester functionality, which can potentially shift the equilibrium via transthioesterification to obtain a cyclic product with low reactivity.

To enhance the ligation of [GPP]_x peptides, the length of the TREN–(suc–OH)₃ arms was increased by adding an intermediate peptide segment that included a structure-disrupting switch (Figure 1). For this purpose, the peptide sequence GPPGPPG(Ψ)SPGPP (I) was synthesized on a solid support (see Scheme 1 and the Supporting Information). The DEPSI segment (G7(Ψ)S8) was constructed by coupling Boc–Ser(OH)–OH that exhibits an unprotected β -hydroxyl side chain followed by the subsequent coupling of Fmoc–Gly OH to this β -OH-group. On-resin synthesis was performed to tether TREN–(suc–OH)₃ to the N-terminal amino functionalities of the three supported peptides (I on support). After the liberation from the support and purification, III was isolated. In III, the TREN core is connected to three homosymmetric CMP strands, in which each presents single structure disrupting switch defects (Scheme 1). The C-terminal carboxylate of each strand of the templated peptides was transformed quantitatively into thioester moieties using *p*-acetamidothiophenol. The resulting IV was further ligated with CPP[GPP]₄ strands under the NCL conditions, which required the Pro←Cys ligation side.^[56] However, the Gly1-to-Cys1 exchange likely induces a weaker effect on CTH propensity than the N-terminal extension of [GPP]_x with an additional Cys.



Scheme 1. Synthesis of the template-preorganized CMPs containing one switch peptide segment per peptide strand. A) Solid-phase-supported peptide synthesis that introduces switch defects into the primary peptide strand I. B) On-resin coupling of the TREN scaffold, which contains three carboxy functionalities with I. C) Liberation of the scaffold-bonded peptides from the acid labile 2-chlorotrityl resin under slightly acidic conditions that retain the Boc-protection of the DEPSI moieties. D) Incorporation of the active thioester functionality to the C-terminal COOH functionality using *p*-acetamidothiophenol. E) NCL of construct IV and cysteine-bearing peptides CPP[GPP]₄. F) Final deprotection of ligation product IV, which contains DEPSI defect structures enabling the pH-activable release of the switch and activation of the triple helical structure formation of the TREN-template-tethered (GPPGPPGSPGPP–CPP[GPP]₄)₃ peptides.

In case of substitution, the repetitive sequence pattern in the CPP[GPP]₄ strands was most likely less disturbed,^[57] if compared to the alternative N-terminal extension, where an additional Cys residue would break the motif of the template-[GPP]_x–Cys-[GPP]_y. However, it should be mentioned that replacing Gly or Pro in CMP will have an effect on the CTH formation kinetics and stability.^[22] Although there are techniques available, e.g., traceless Staudinger ligation, which allows Cys-free ligation, the NCL was chosen as it provides a suitable strategy to utilize recombinantly expressed CPP[GPP]_x strands or other recombinant collagens.^[58] NCL was most effectively performed under denaturation conditions, where the utilized buffer included guanidinium hydrochloride (Gdn-HCl, 6 M), tris(2-carboxyethyl)phosphine (TCEP) as a reducing agent, and sodium ascorbate serving as a radical scavenger without any thiols such as sodium 2-mercaptoethanesulfonate. The obtained product TREN–(suc–GPPGPPG(Ψ)SPGPP–CPP[GPP]₄)₃ (Scheme 1, VI) contained a total of nine “GPP” repetitions per arm with switch segments at position 8 and G→C substitutions at position 13. Compound VI was purified by chromatography, and the chemical identity was verified by UPLC–MS. In the final step, the obtained CMP was fully deprotected and stored under slightly acidic conditions (pH = 5) to keep the switch fully protonated and,

thus, preserve the disturbed state (Scheme 1, VII, CMP^(+T+S)). The sample set was complemented with appropriate controls, such as the template-tethered peptides without a switch segment (TREN–(suc–GPPGPPGSPGPP–CPP[GPP]₄)₃ VIII, CMP^(+T–S)) and nontemplate attached peptides with and without switch segments (H₂N–GPPGPPG(Ψ)SPGPP–CPP[GPP]₄ IX, CMP^(–T+S) and H₂N–GPPGPPGSPGPP–CPP[GPP]₄ X, CMP^(–T–S), respectively; see Figure 2c and the Supporting Information).

Both switch systems (VII and IX) were well soluble and exhibited structural stability at pH = 5.0. The slight increase in pH to 6.2 or higher triggered a well-controlled O→N-acyl transfer rearrangement with increasing rates at higher pH values.^[45,48,51,52] For instance, a *t*_{1/2} of 1 min was reported for the O–N-intramolecular acyl migration in amyloid Aβ1-42 peptides at pH = 7.4.^[48] Hence, it is necessary to clarify whether both the template and switch influence the CTH formation in the prepared CMPs. The ability of the peptide constructs to adopt a triple helical structure was investigated by circular dichroism (CD) far-ultraviolet spectroscopy in solution, which represented a commonly used method for the CTH fold analysis of CMPs.

The template-tethered peptides with and without switch segments (VII, CMP^(+T+S) and VIII, CMP^(+T–S), respectively) as well as the nontemplated peptide strand controls with and without

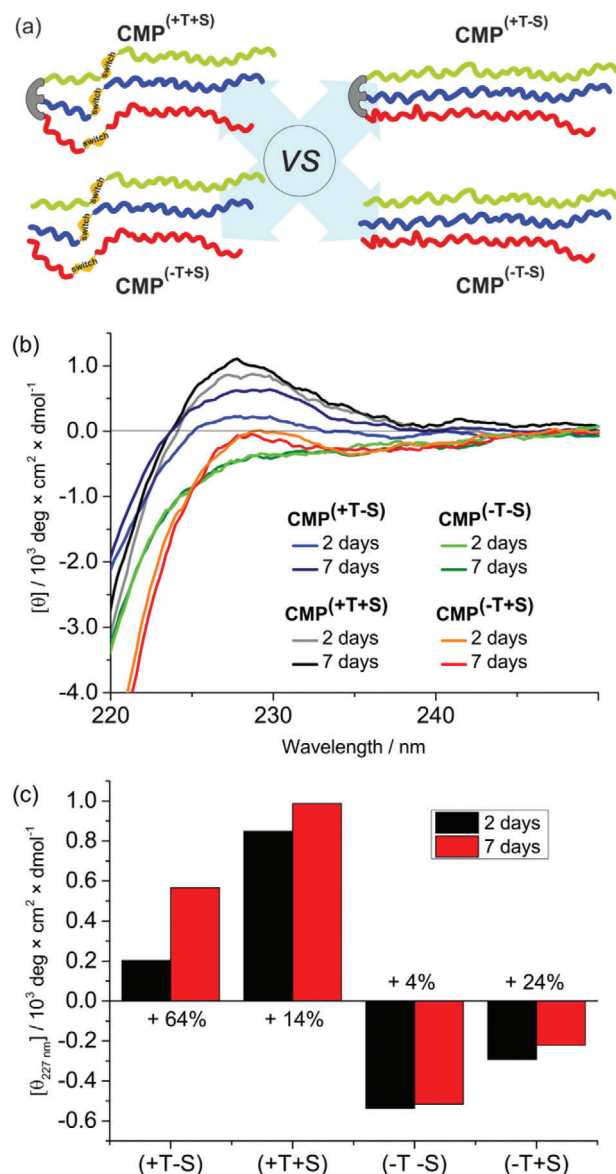


Figure 2. a) Schematic illustration of the CMPs with and without templates as well as the CMPs with and without switch structure-disturbing defects. b) CD spectra of the CMPs equilibrated at pH = 7.4 for 2 and 7 days. c) Ellipticity changes at 227 nm used as a relevant indicator of the CTH formation process. Conditions: 0.2 mg mL^{-1} in $100 \times 10^{-3} \text{ M}$ phosphate buffer at 4°C .

switch segments (IX, CMP^(-T+S) and X, CMP^(-T-S)), respectively) were incubated at pH = 7.4 at a concentration of 10 mg mL^{-1} (4°C) for 2 days. Aliquots of these solutions were taken and diluted to 0.2 mg mL^{-1} to perform CD measurements after 2 and 7 days (Figure 2a). Notably, the CD spectra of all template-containing CMPs clearly exhibit a positive Cotton effect at $\approx 227 \text{ nm}$ (Figure 2a), which was attributed to the formation of triple helical structures in previous congener CMP/collagen studies.^[30] In contrast, all nontemplate-tethered control CMPs possessed negative CD values regardless of the measurement time or the presence of a switch segment. These findings highlight a distinct effect of

the template on CTH formation (Figure 2a). The introduction of a switch domain affects the templated CMPs and notably contributes to the adaptation of a triple helix motif. However, in the nontemplated system, the switch domain seems to have a positive effect on shifting the structure in the triple helix direction, as indicated by the observed increase in the CD signal intensities at $\approx 227 \text{ nm}$ (Figure 2a; CMP^(+T-S) \rightarrow CMP^(+T+S) and CMP^(-T-S) \rightarrow CMP^(-T+S)).

The quantitative evaluation of CTH development with time during incubation at pH = 7.4 revealed the discrete effects of both the template and the switch domains (Figure 2b). The template-tethered peptides (CMP^(+T+S) and (+T-S)) demonstrated the highest changes in ellipticity over time. CMP^(+T+S) with the template and switches achieved the highest molar ellipticity $[\theta_{\text{max}(+T+S)}] = 1.0 \times 10^3 \text{ deg cm}^2 \text{ dmol}^{-1}$ and exhibited a 14% ellipticity increase between 2 and 7 days of incubation. In comparison, CMP^(+T-S) containing the template without structure-disturbing switches reached $[\theta_{\text{max}(+T-S)}] = 0.58 \times 10^3 \text{ deg cm}^2 \text{ dmol}^{-1}$ after 7 days of incubation, but demonstrated a 64% ellipticity increase between days 2 and 7. Apparently, the introduced switch segments changed the peptide structural dynamics, enabling the three peptide strands to adopt the CTH structure motif during the initial 2 days without further structural optimization, which was not evident until day 7. This explanation appears reasonable as a rapid O \rightarrow N-acyl transfer rearrangement can be expected at pH = 7.4,^[48] accelerating the switching process to proceed much higher compared to the first 2 days of incubation. It can be hypothesized that in the absence of structure-disturbing switches, the template-tethered peptide strands associate in an ill-defined manner and only slowly overcome the energetic barriers to fold into the desired CTH motif. The maximum molar ellipticity of CMP^(+T+S) was $[\theta_{\text{max}(+T+S)}] = 1.0 \times 10^3 \text{ deg cm}^2 \text{ dmol}^{-1}$, which was comparable to that of the congener constructs described in the literature.^[26,37] These results are very intriguing, considering the relatively short peptide sequences of the nine “GPP” repeating units and noncollagenous residues (Cys and Ser). The latter are categorized to not favor the CTH formation as they may affect ring puckering, interstrand H-bonds, and steric repulsion between the three strands, which constitute important factors strongly influencing CTH stability.^[27,38,53,59–65]

No evidence for a triple helical structure was observed for both control peptide strands without a template (CMP^(-T-S) and (-T+S)) as these samples possessed negative mean residue ellipticity values at 227 nm after 2 and 7 days of incubation. These controls exhibited constant ellipticity values over time within the experimental error, suggesting that the rapid formation of local minimum structures and CTH formation is by any means not favored. This highlights the importance of the organic template in preorganizing the CTH motif and reduces the formation of ill-defined aggregation. Interestingly, the switch defect segment produced a negligible effect on the peptide organization, in which the strands were not bound to the template. Although CMP^(-T+S) possessed a slightly higher ellipticity than CMP^(-T-S), its value remained negative. These findings indicate that the template anchors the peptide strands with an appropriate geometry and allows reaching their high local concentration to promote intramolecular folding by reducing entropic penalties.^[37] The switch defects, however, suppress the initial ill-defined association of peptide strands despite the template and, thus, promote the formation

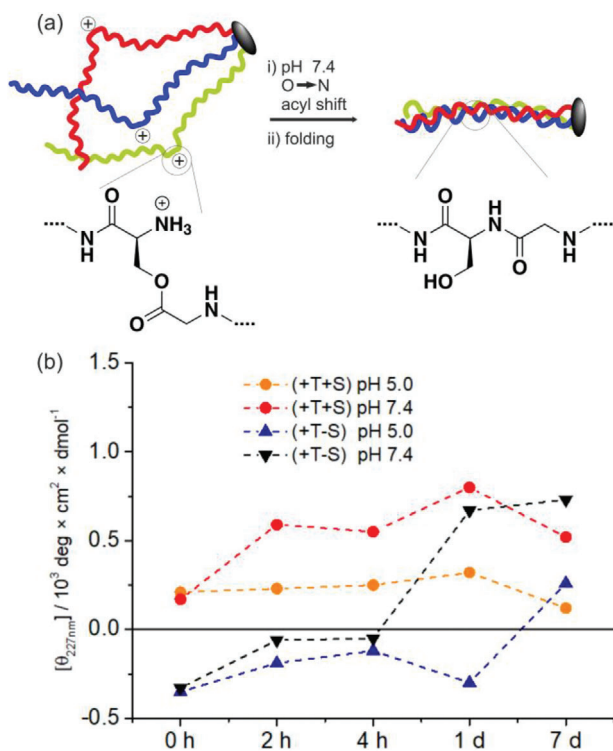


Figure 3. pH jump experiments investigating the effect of the pH-triggered switch on the collagen-like triple helix formation. a) Schematic illustration of the switch event that triggers the formation of an undisturbed CTH. b) Mean residue ellipticity values obtained at pH values of 5.0 and 7.4 by CD spectroscopy and plotted as a function of the equilibration time for the switchable and nonswitchable CMPs. Conditions: CMP incubation at 10 mg mL^{-1} for 1 day (4°C) and dilution to 0.2 mg mL^{-1} with $100 \times 10^{-3} \text{ M}$ buffer at a pH of 5.0 or 7.4 ($t = 0$) and at a temperature of 4°C . The lines should be used only as visual guides.

of a collagen-like triple helix by enhancing the early structural dynamics.^[37]

The impact of the switching process on the peptide organization in templated CMPs is evident, although the expected rates of the switch event at $\text{pH} = 7.2$ appear to be considerably faster (minutes) than the duration of the overall peptide structural optimization (hours or days). To provide further insights into the exact timing of the helix formation process, pH jump experiments were performed (Figure 3). In these experiments, the template-tethered peptides with and without switch segments (CMP^(+T+S) and CMP^(+T-S)) were separately equilibrated for 1 day at $\text{pH} = 5.0$. The samples were then diluted with the buffer from 10 to 0.2 mg mL^{-1} to achieve the final pH values of 5.0 or 7.4 (Figure 3, $t = 0$ h). The resulting solutions were investigated by CD spectroscopy to compare their ellipticity at 227 nm (Figure 3). In fact, the CMP^(+T+S) samples with $\text{pH} = 5.0$ and $\text{pH} = 7.4$ exhibited higher molar ellipticity values at $t = 0$ h than the corresponding controls (CMP^(+T-S)) with the same pH values. This observation confirmed that the CTH folding of the CMPs was generally assisted by the switch defects. Consistent with the obtained data, the ellipticity of the switch-containing sample CMP^(+T+S) increased immediately after raising the pH to 7.4 and leveled off within the next 2 h at $[\theta_{227\text{nm}}] \approx 0.6 \times 10^3 \text{ deg}$

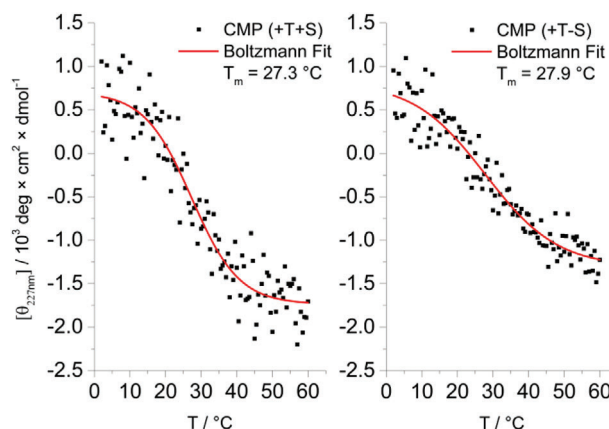


Figure 4. CD-spectroscopy-monitored denaturation of the peptide structures formed via the pH-induced folding of CMP^(+T+S) (left) and CMP^(+T-S) (right) after 5 day equilibration. Conditions: CMP incubation at 10 mg mL^{-1} for 2 days (4°C , $100 \times 10^{-3} \text{ M}$ buffer, $\text{pH} = 7.4$), dilution to 0.2 mg mL^{-1} , and equilibration for 5 days prior to measurements; heating rate: 1°C min^{-1} ; Boltzmann fit of all data points ($R^2(\text{CMP}^{(+T+S)}) = 0.913$ and $R^2(\text{CMP}^{(+T-S)}) = 0.918$); inflection points are assigned to T_m .

$\text{cm}^2 \text{ dmol}^{-1}$. However, CMP^(+T+S) at $\text{pH} = 5.0$ demonstrated no significant ellipticity increase over the 7 day period within the experimental error, and its ellipticity remained low at $[\theta_{227\text{nm}}] = 0.12 \times 10^3 \text{ deg cm}^2 \text{ dmol}^{-1}$.

While the molar ellipticity of CMP^(+T+S) rapidly increased within 2 h after the pH jump, the CMP^(+T-S) sample demonstrated a significant increase in ellipticity only after 1 day. However, both the CMP^(+T+S) and CMP^(+T-S) samples ultimately achieved similar molar ellipticity values at $\text{pH} = 7.4$, indicating that the introduced template facilitates the preorganization process and assists in adopting the CTH motif. However, the switch apparently provides sufficient dynamics at the early stage to more rapidly adopt the triple helical structure. Although a similar motif can be adopted in the absence of switch defects, the folding kinetics were slower in the case of CMP^(+T-S), indicating a less dynamic initial state. Interestingly, these experiments suggest that the CTH of the templated CMPs exists most likely in the state of a thermodynamic equilibrium, as the same structure can be reached from the two different initial states regardless of the starting point CMP^(+T+S) or CMP^(+T-S).^[46,66,67]

To exclude that the increase in ellipticity is not only related to the change of pH, the pH jump experiment was repeated for the nonswitchable control CMP^(+T-S) under the same conditions (Figure 3). Although the results of pH jump experiments clearly revealed the existence of a positive effect of the switch on CTH formation, it can be assumed that the equilibration at $\text{pH} = 5.0$ does not favor discrete folding. As expected, the obtained CD spectra show that the ellipticity values obtained at $\text{pH} = 5.0$ are lower than those at $\text{pH} = 7.4$. However, at $\text{pH} = 5.0$, both the CMP^(+T+S) and CMP^(+T-S) samples converge at rather similar ellipticity values of $[\theta_{227\text{nm}}] \approx 0.2 \times 10^3 \text{ deg cm}^2 \text{ dmol}^{-1}$.

Temperature-dependent CD spectroscopy was conducted to monitor the “melting” of different triple helical structures (Figure 4). Their ellipticity at 227 nm was measured in the temperature range of 0 – 60°C at a moderate heating ramp of 1 K min^{-1} and then fitted with the Boltzmann function. The short



CMP strands and moderate heating rates produced relatively noisy ellipticity data that did not allow us to reveal quantitative degrees of cooperativity of the unfolding processes. However, the Boltzmann fits reached a coefficient of determination (R^2) of 0.91–0.92, suggesting that the regression model fits the observed data very well (Supporting Information). Thus, structural transition temperatures of CMP^(+T+S) and CMP^(+T-S) were estimated to be within the method accuracy rather similar, resulting in melting temperature (T_m) values of 27.3 and 27.9 °C, respectively. These temperatures were between the melting temperatures of the non-preorganized [GPP]₁₀ (T_m = 23 °C) and preorganized TREN-[suc-(Gly-Nleu-Pro)₆-NH₂]₃ (T_m = 46 °C) CMP constructs reported in the literature.^[4,37] This not only indicated the formation of a more stable CTH motif of the templated CMPs compared to that of the nontemplated [GPP]₁₀ strands, but also confirmed the presence of the above-discussed structure-disturbing effects of Cys and Ser point mutations in [GPP]_x strands.^[2,4,37,68] Moreover, the obtained melting curves had rather different slopes, suggesting that a more cooperative unfolding process occurred in the case of CMP^(+T+S) as compared with that of CMP^(+T-S). The stronger cooperative effect observed for the CMP^(+T+S) melting process would validate the hypothesis stating that the formed switch defects contribute to the perfection of the CTH structural motif.

It should be emphasized that despite the CD results indicating the formation of CTH, transmission electron microscopy or atomic force microscopy images were not revealing higher level of organization in any of the CMPs. This might have been expected as the aspect ratio of the nano-objects that is required for nematic phase segregation is in the present systems rather low. Moreover, it might be speculated that the template disturbs the packing of the CTHs into bundles. These might be overcome, if the length of the peptide segments will be increased, and potentially a concept of sticky ends might be introduced.

3. Conclusions

In this study, a TREN-(suc-GPPGPPGSPGPP-thioester)₃ scaffold was synthesized to enable the coupling of three CPP[GPP]_x strands and obtain monodispersed CMPs via native chemical ligation. Additional DEPSI peptide units (switch defects) were incorporated into the peptide segments close to the template to allow the pH-triggered formation of the collagen triple helices (CTHs). The scaffold-tethered constructs with and without switches as well as the corresponding control samples of the nontemplated peptide strands were compared by CD spectroscopy. Both the organic scaffold and switch defects produced significant effects on the CTH motif formation, as indicated by the results of the 7 day equilibration experiments conducted under constant pH conditions, by pH jump experiments, and by CTH “melting” studies. The equilibration performed at pH = 5 hindered the folding of the peptide strands into a CTH; however, after increasing the pH to 7.4, the switch-containing constructs rapidly adopted a CTH motif. The temperature-dependent CD spectroscopy data confirmed the general assumption that the switch defects obviously contributed to the formation of a more stable CTH motif by enhancing the structural dynamics at the early stages of the folding process. The proposed strategy may pave the way for the development of CMP constructs with higher molecular weights

by utilizing recombinantly expressed CPP[GPP]_x strands or other recombinant collagens.^[58] These might result in CMPs, which were less limited in terms of the number of repeating units than the chemically synthesized CPP[GPP]_x strands presented in this proof-of-principle study and thus would probably offer access to materials with properties closer to naturally occurring collagen.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

collagen mimetic peptides, collagen triple helix, self-organization

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